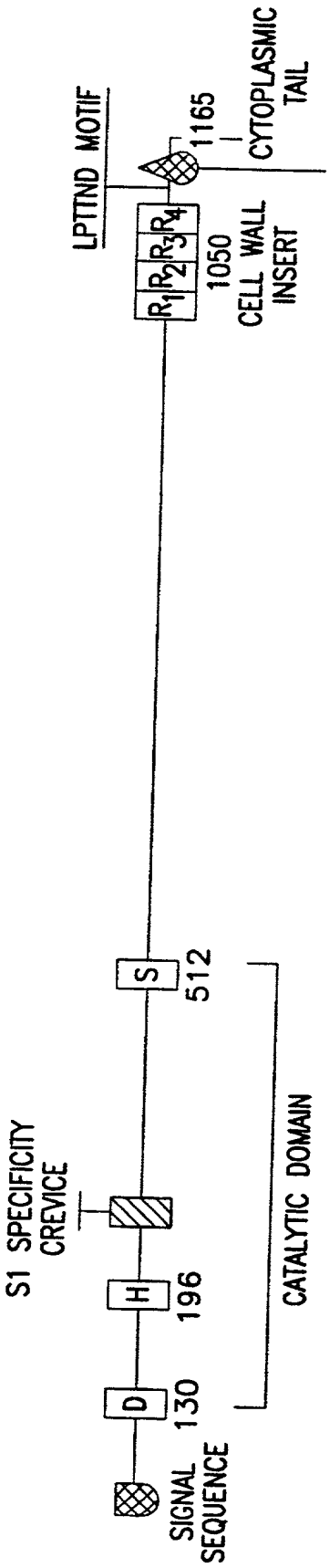
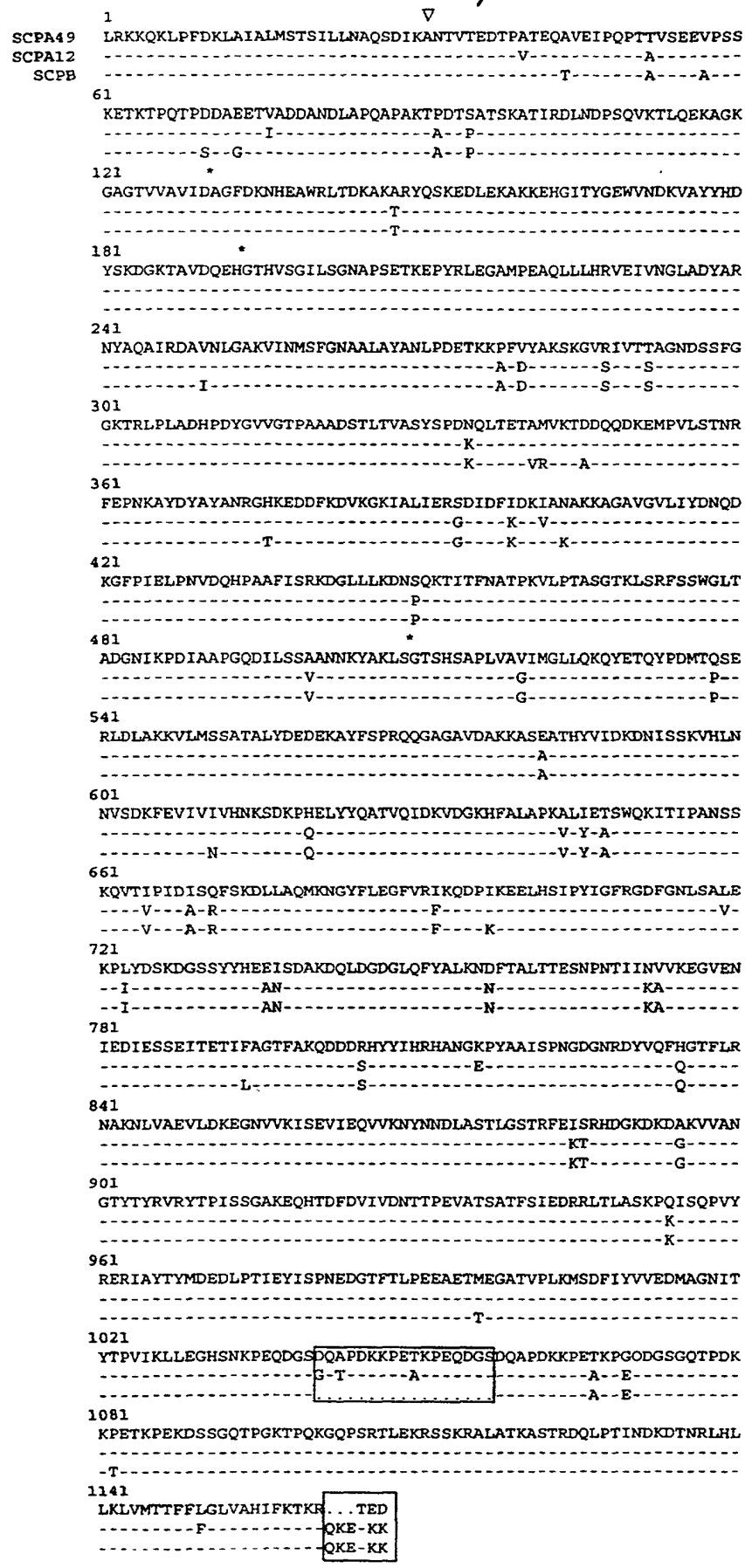

SIGNAL SEQUENCE
D
130
H
196
S1 SPECIFICITY CREVICE
S
512
CATALYTIC DOMAIN
R1 R2 R3 R4
1050
CELL WALL INSERT
LPTTND MOTIF
1165
CYTOPLASMIC TAIL
MEMBRANCE ANCHOR

FIG. 1

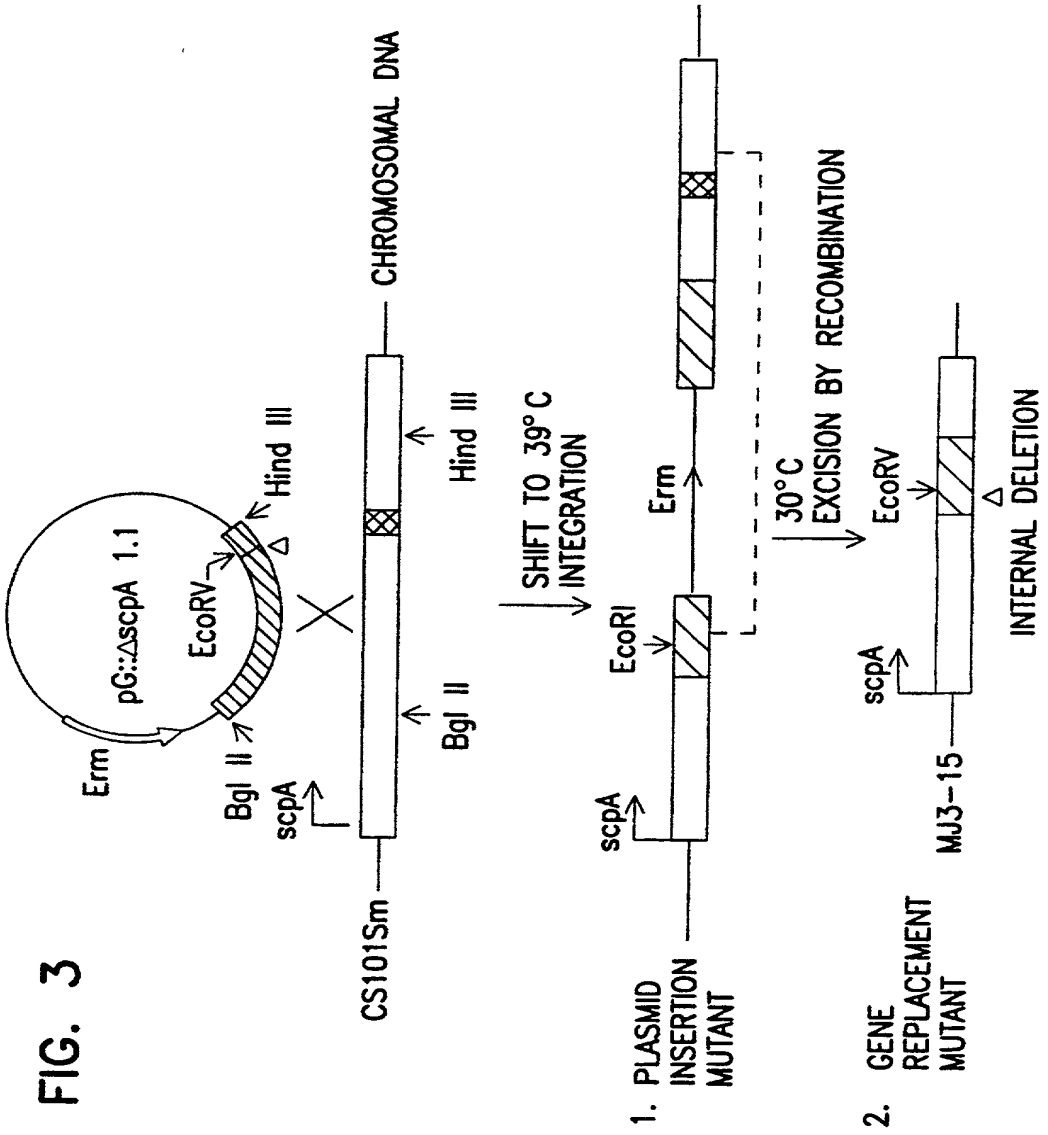

1
SCPA49 LRKKQKLPFDKLAIALMSTSILLNAQSDIKANTVTEDTPATEQAVEIPQPTTVSEEVPSS
SCPA12 ----------V----------A--------
SCPB ----------T-------A----A---
61
KETKTPQTPDDAEETVADDANDLAPQAPAKTPDTSATSKATIRDLNDPSQVKTLQEKAGK
----------I----------A--P----------
---------S--G----------A--P----------
121
GAGTVVAVIDAGFDKNHEAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWVNDKVAYYHD
----------T----------
----------T----------
181
YSKDGKTAVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLHRVEIVNGLADYAR
241
NYAQAIRDAVNLGAKVINMSFGNAALAYANLPDETKKPFVYAKSKGVRIVTTAGNDSSFG
----------A-D-------S---S--------
---------I----------A-D-------S---S--------
301
GKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDNQLTETAMVKTDDQQDKEMPVLSTNR
----------K----------
----------K-----VR---A----------
361
FEPNKAYDYAYANRGHKEDDFKDVKGKIALIERSDIDFIDKIANAKKAGAVGVLIYDNQD
----------G----K--V----------
----------T----------G----K----K----------
421
KGFPIELPNVDQHPAAFISRKDGLLLKDNSQKTITFNATPKVLPTASGTKLSRFSSWGLT
----------P----------
----------P----------
481
ADGNIKPDIAAPGQDILSSAANNKYAKLSGTSHSAPLVAVIMGLLQKQYETQYPDMTQSE
----------V----------G----------P--
----------V----------G----------P--
541
RLDLAKKVLMSSATALYDEDEKAYFSPRQQGAGAVDAKKASEATHYVIDKDNISSKVHLN
----------A----------
----------A----------
601
NVSDKFEVIVIVHNKSDKPHELYYQATVQIDKVDGKHFALAPKALIETSWQKITIPANSS
----------Q----------V-Y-A----------
----------N--------Q----------V-Y-A----------
661
KQVTIPIDISQFSKDLLAQMKNGYFLEGFVRIKQDPIKEELHSIPYIGFRGDFGNLSALE
----V---A-R----------F----------V-
----V---A-R----------F----K----------
721
KPLYDSKDGSSYYHEEISDAKDQLDGDGLQFYALKNDFTALITTESNPNTIINVVKEGVEN
--I----------AN----------N----------KA-------
--I----------AN----------N----------KA-------
781
IEDIESSEITETIFAGTFAKQDDDRHYYIHRHANGKPYAAISPNGDGNRDYVQFHGTFLR
----------S----------E----------Q-----
----------L----------S----------Q-----
841
NAKNLVAEVLDKEGNVVKISEVIEQVVKNYNNDLASTLGSTRFEISRHDGKDKDAKVVAN
----------KT--------G-----
----------KT--------G-----
901
GTYTYRVRYTPISSGAKEQHTDFDVIVDNTTPEVATSATFSIEDRRLTLASKPQISQPVY
----------K------
----------K------
961
RERIAYTYMDEDLPTIEYISPNEDGTFTLPEEAETMEGATVPLKMSDFIYVVEDMAGNIT

----------T----------
1021
YTPVIKLLEGHSNKPEQDGSDQAPDKKPETKPEQDGSDQAPDKKPETKPGODGSGQTPDK
----------G-T------A----------A--E----------
----------A--E----------
1081
KPETKPEKDSSGQTPGKTPQKGQPSRTLEKRSSKRALATKASTRDQLPTINDKDTNRLHL

-T----------
1141
LKLVMTTFFLGLVAHIFKTKR...TED
---------F----------QKE-KK
----------QKE-KK

FIG. 2

FIG. 3


Erm
pG::ΔscpA 1.1
EcoRV
Hind III
Bgl II
scpA
CS101Sm
Bgl II
Hind III
CHROMOSOMAL DNA
SHIFT TO 39° C
INTEGRATION
scpA
EcoRI
Erm
1. PLASMID INSERTION MUTANT
30°C
EXCISION BY RECOMBINATION
scpA
EcoRV
MJ3-15
INTERNAL DELETION
2. GENE REPLACEMENT MUTANT

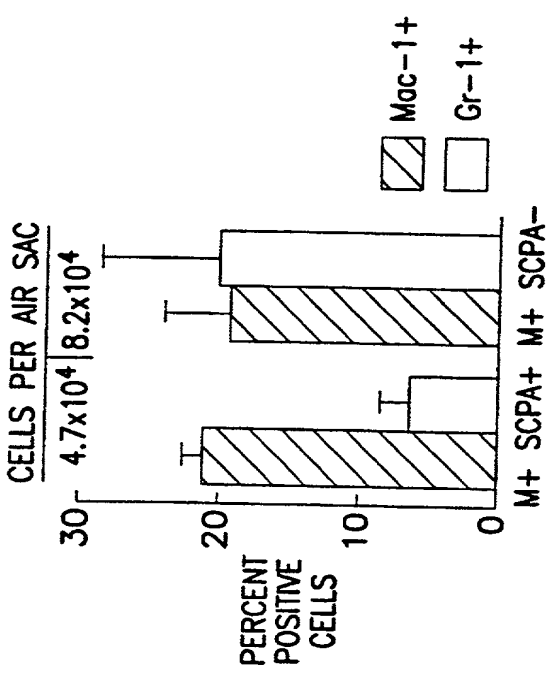
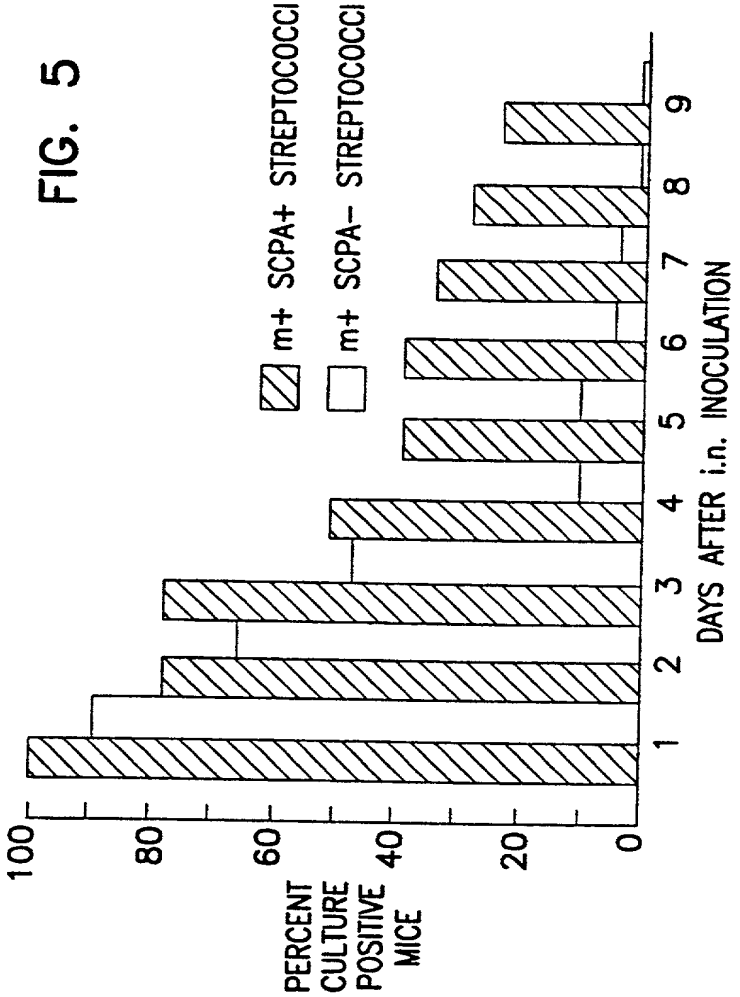

CELLS PER AIR SAC
4.7x10⁴
8.2x10⁴
30
20
10
0
PERCENT POSITIVE CELLS
Mac-1+
Gr-1+
M+ SCPA+
M+ SCPA-

FIG. 4

FIG. 5


m+ SCPA+ STREPTOCOCCI
m+ SCPA- STREPTOCOCCI
PERCENT CULTURE POSITIVE MICE
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
DAYS AFTER i.n. INOCULATION

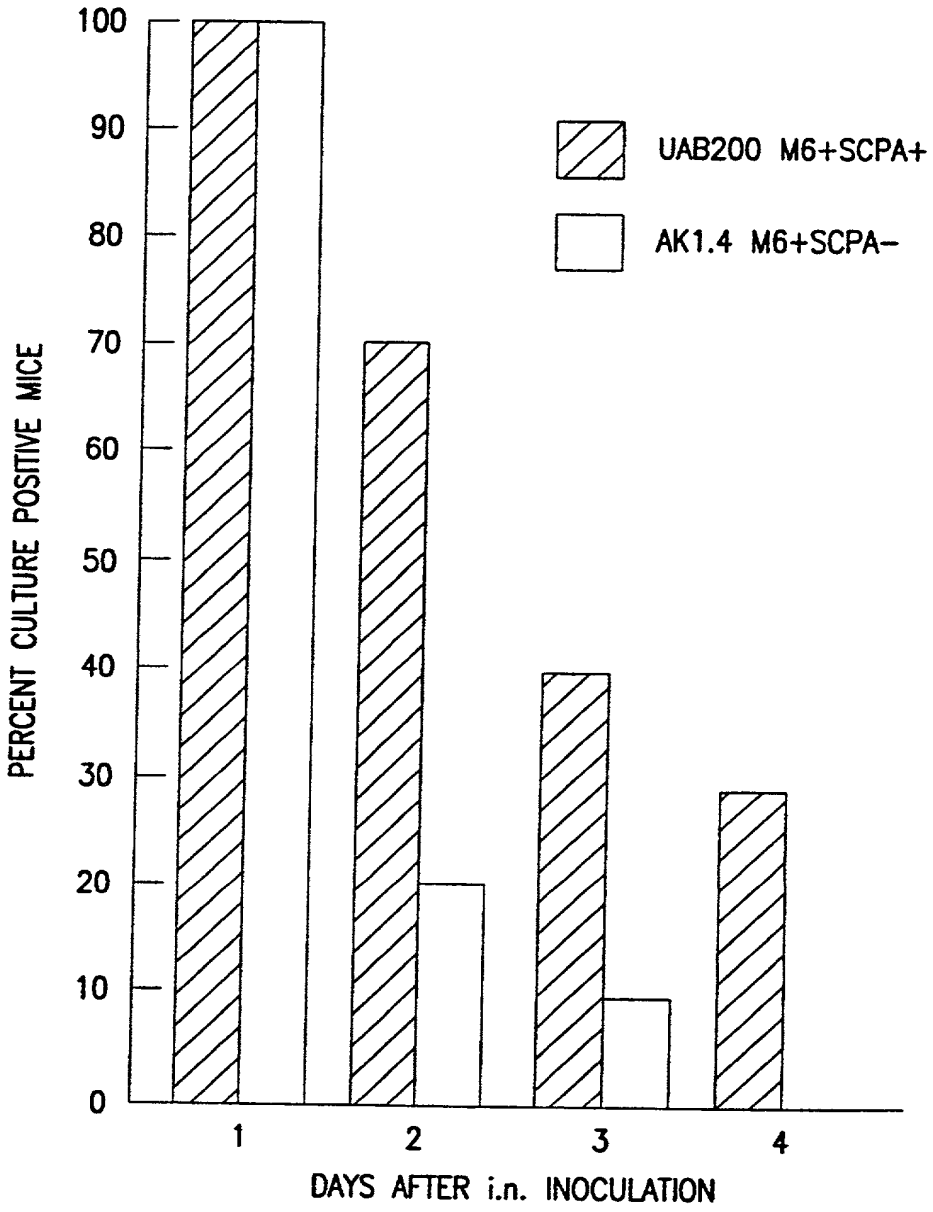

100
90
80
70
60
50
40
30
20
10
0
PERCENT CULTURE POSITIVE MICE
1
2
3
4
DAYS AFTER i.n. INOCULATION
UAB200 M6+SCPA+
AK1.4 M6+SCPA-

FIG. 6

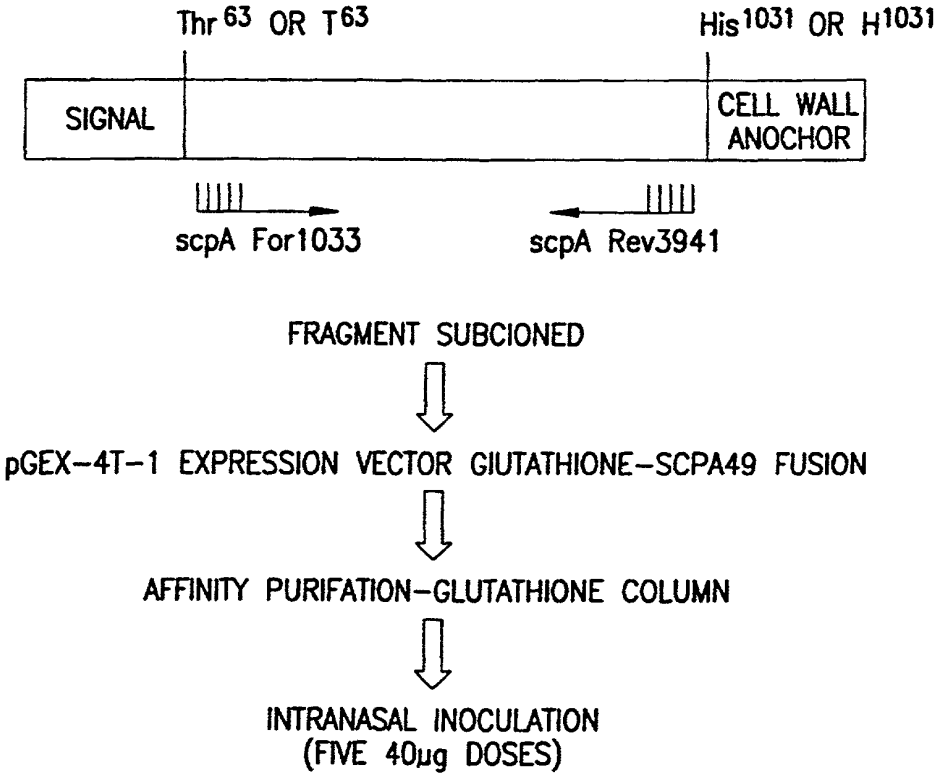

Thr 63 OR T63
His1031 OR H1031
SIGNAL
CELL WALL ANOCHOR
scpA For1033
scpA Rev3941
FRAGMENT SUBCIONED
pGEX-4T-1 EXPRESSION VECTOR GIUTATHIONE-SCPA49 FUSION
AFFINITY PURIFATION-GLUTATHIONE COLUMN
INTRANASAL INOCULATION
(FIVE 40µg DOSES)

FIG. 7

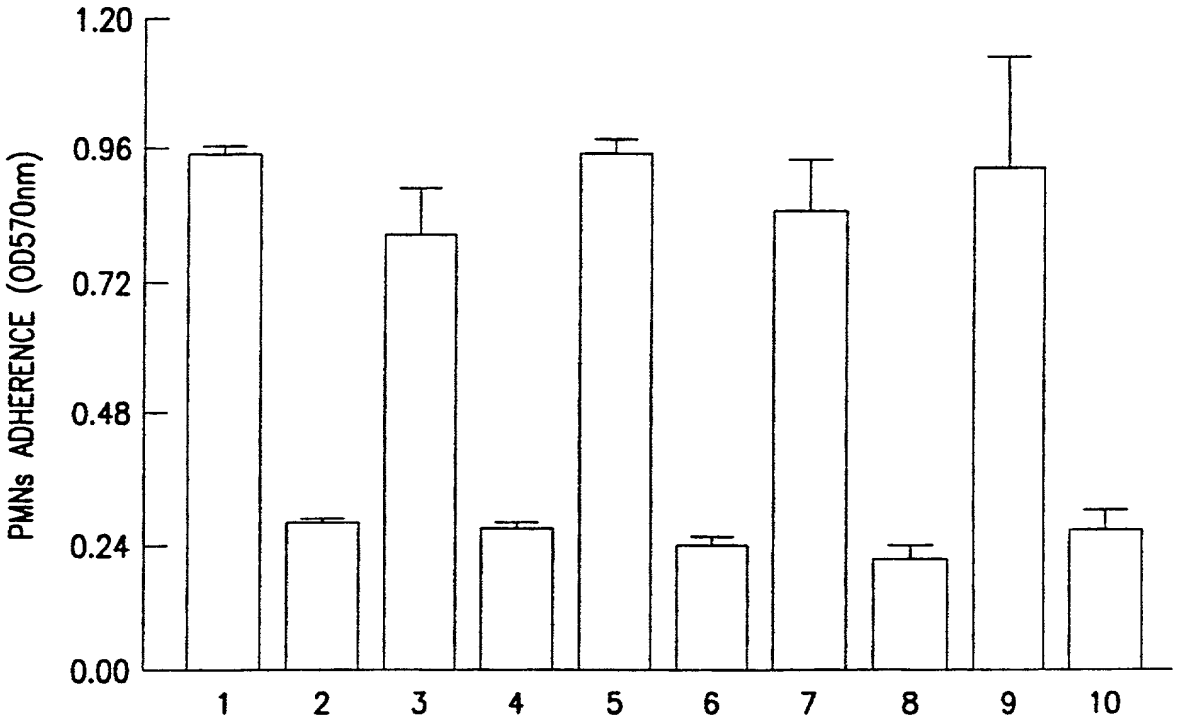


FIG. 8

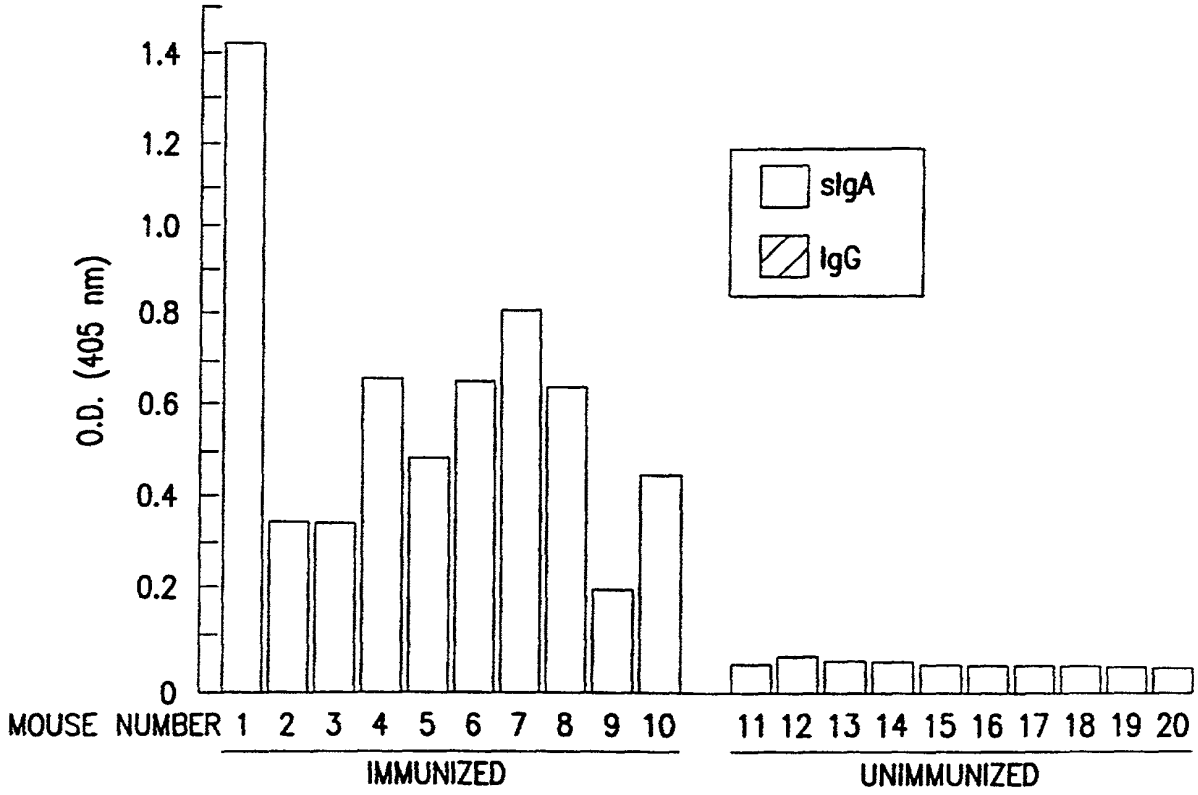

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
O.D. (405 nm)
sIgA
IgG
MOUSE NUMBER 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
IMMUNIZED
UNIMMUNIZED

FIG. 9A

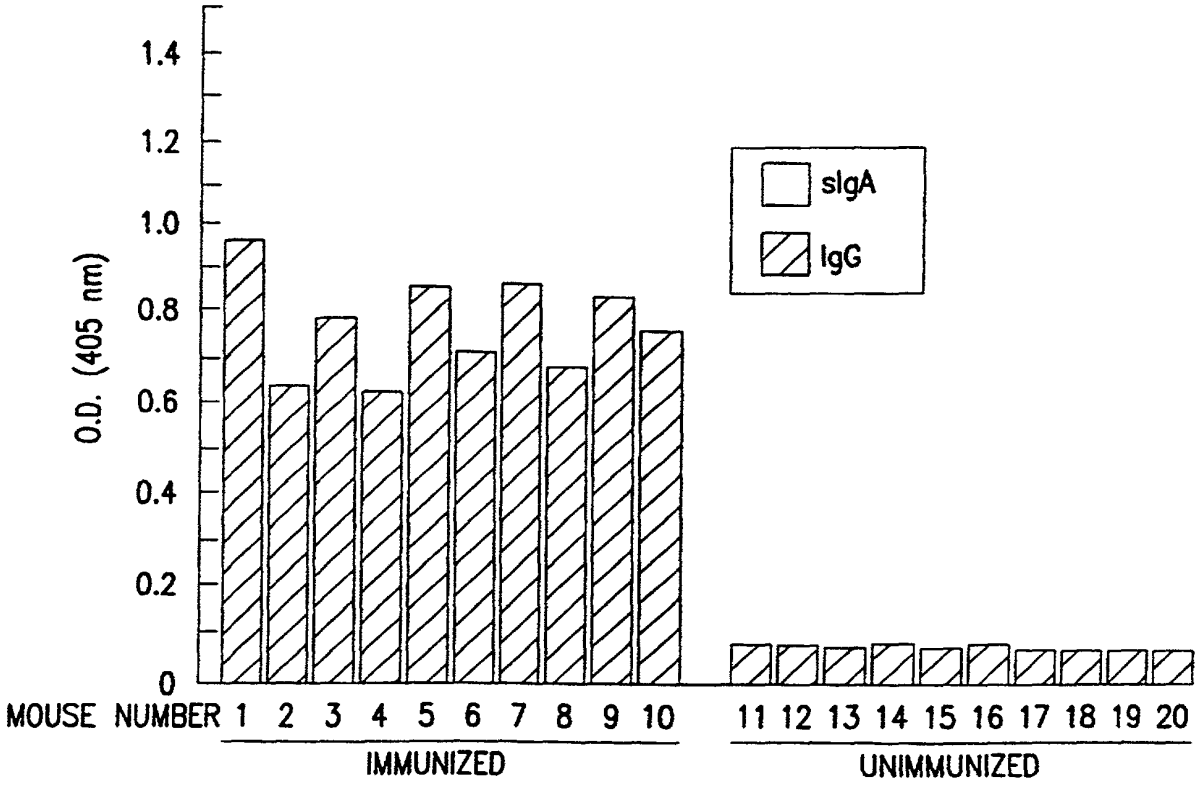

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
O.D. (405 nm)
sIgA
IgG
MOUSE NUMBER 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
IMMUNIZED
UNIMMUNIZED

FIG. 9B

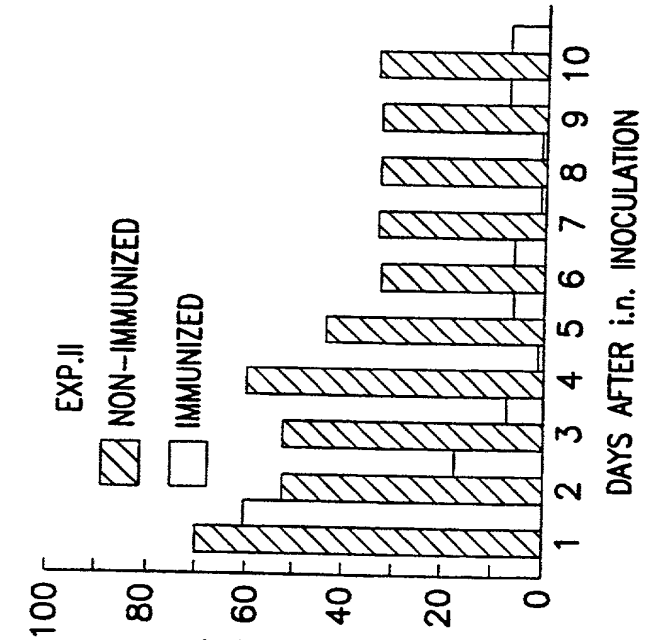


FIG. 10B

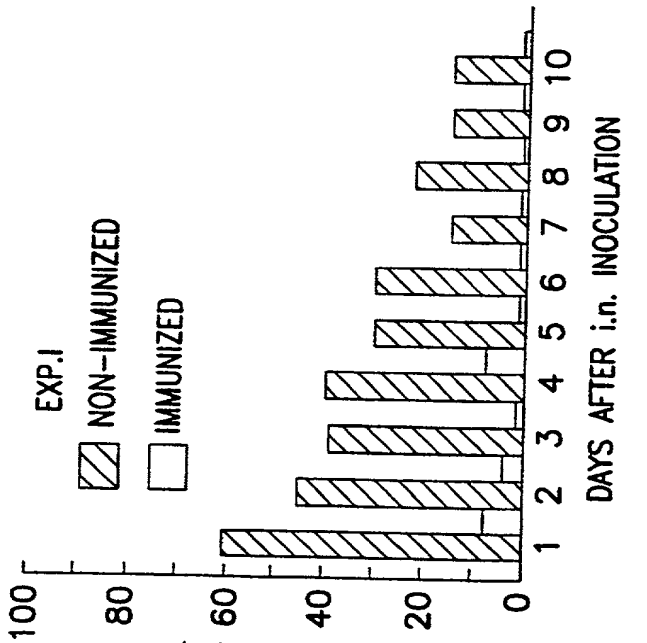


FIG. 10A

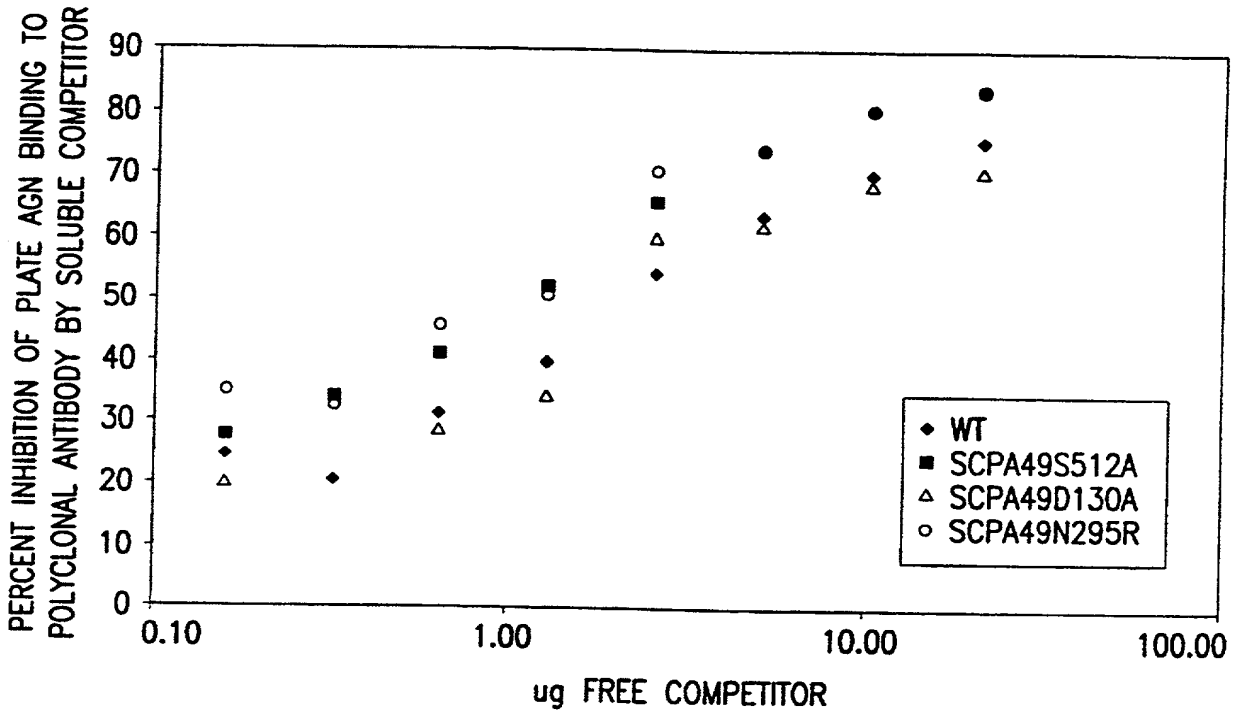

PERCENT INHIBITION OF PLATE AGN BINDING TO POLYCLONAL ANTIBODY BY SOLUBLE COMPETITOR
90
80
70
60
50
40
30
20
10
0
0.10
1.00
10.00
100.00
ug FREE COMPETITOR
WT
SCPA49S512A
SCPA49D130A
SCPA49N295R

FIG. 11

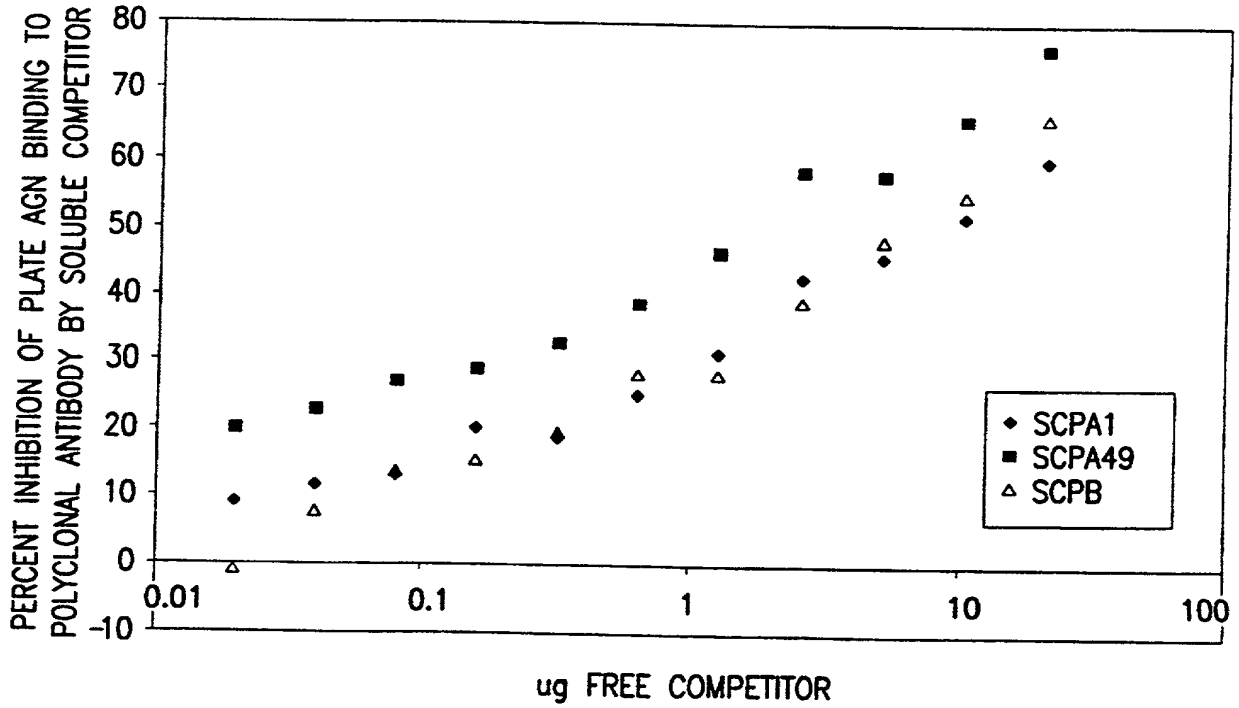

PERCENT INHIBITION OF PLATE AGN BINDING TO POLYCLONAL ANTIBODY BY SOLUBLE COMPETITOR
80
70
60
50
40
30
20
10
0
-10
0.01
0.1
1
10
100
ug FREE COMPETITOR
SCPA1
SCPA49
SCPB

FIG. 12